ORAL ABSTRACTS

FUNGAL MECHANISMS OF TEXTILE DYES BIODEGRADATION
Nelson Lima, Cristiane A. Ottoni, Luis Lima, Cledir Santos
iBB – Institute for Biotechnology and Bioengineering,
Biological Engineering Centre, University of Minho, Braga,
Portugal
Reactive dyes are widely used in the textile industry. Coloured
effluents from dyestuff and textile industries, the major
producers and users of azo dyes, not only produce visual
pollution but can also be detrimental to life, as they are
usually resistant to biological treatment. Additionally, fungi,
mainly white rot fungi, have shown the ability to degrade
numerous aromatic organopolutants, including textile dyes,
via oxidative mechanisms till their complete mineralisation,
avoiding the formation of azolines as intermediates. In our
work, textile azo dyes were synthesised using amino-benzoic
and aminosulfonic acids as diaz components and
bioaccessible groups such as 2-methoxyphenol (guaiaicol),
and 2,6-dimethoxyphenol (syringol) as coupling components.
The bioaccessible groups are present in the lignin structure
and seem to be access points to the ligninolytic enzymes
produced by white rot fungi. The fungal biodegradation of the
azo dyes were studied in order to establish the relationship
between the chemical structure of the dye and the extent of
biodegradation. The rule of the non-specific fungal ligninolytic
enzymatic system, lignin peroxidases, manganese peroxidases
and laccases, as well as the enzyme glycocalix oxidade with
produce H2O2 for the activities of both peroxidases were
studied. Reactive Black 5 and the aztriquinone-based
polymeric dye Poly R-745 have been currently used to screen
the fungal biodegradation under alkaline conditions (pH =
8.0). In order to adapt the fungi to this alkaline condition
a chemostat is now used. To perform this work the fungi
used were supplied by the culture collection Micoteca da
Universidade do Minho (MUM).

FUNCTIONAL GENOME ANALYSIS OF R. EUTROPHA AS A
BASIS FOR HYDROGEN-BASED BIOTECHNOLOGY
Anne Pohlmann1, Yvonne Kohlmann2, Andreas Otto2,
Doerte Becher2, Michael Hecker2, Boerbel Friedrich1
1 Humboldt-Universitaet zu Berlin, Institut fur Biologie/
Mikrobiologie, 10115 Berlin
2 Ernst-Moritz-Arndt-Universitaet Greifswald, Institut fur
Mikrobiologie, 17489 Greifswald
Ralstonia eutropha H16 is a strictly respiratory organism
capable of growing either on a wide range of organic
compounds or in the absence of such substrates, on the
inorganic substances hydrogen and carbon dioxide as
sole sources of energy and carbon, respectively. R. eutropha
is of biotechnological interest (e.g. for the synthesis of
biomolecules labeled with stable isotopes and for the
industrial production of biopolymers) and has an important
potential for application in hydrogen-based technologies.
Metabolic expression profiling monitored by transcript
analyses and proteome studies carried out with cells grown
heterotrophically on the fast growth-promoting substrate
succinate and autotrophically with hydrogen provide insights
in the control of metabolic routes and capacities of the
organism. This knowledge is crucial for the engineering of
novel strains for the production of biomolecules on the basis
of hydrogen. As a key to understand energy and transport
processes, membrane proteins were included in the studies.
These experiments revealed characteristic alterations in the
respiratory chain as well as changes in the pattern of transport
systems. In addition, mutant studies showed the impact of
global regulator systems on hydrogen metabolism.

EXPRESSION OF THE GENES FOR
CONVERSION OF NITRILES AND AMIDES IN
RHODOCCOCUS ERYTHROPILUS
Miroslav Patek, Adam Pavlik, Monika Knoppova,
Ondrej Kaplan, David Kubac, Jan Nevevra,
Ludmila Martinkova, Miroslav Patek
Institute of Microbiology AV CR, v. v. i., Prague, Czech Republic
Enzymes of aldolxime=nitrile=amide=acid metabolic
pathway represent versatile tools in biotechnology.
Nitrilases, nitrile hydratases and amidases from rhodococci
were characterized and applied in large-scale industrial
biotransformations. We isolated the gene cluster containing
and (aldoxime dehydratase), amt (amidase) and
 amt1-nha2 (nha1-nha2 (x- and &-substrates of nitrile hydratase)
from R. erythropolis AT4
with the aim to analyze regulation of their expression and
to manipulate the genes to improve specific activities of
the enzymes. The complete nucleotide sequence of the cluster
(9552 bp, GenBank Acc. No. JQ406017) also covered
the genes nha1, nha2, nha3 and nha4 coding for regulatory proteins.
All genes of the cluster are transcribed in the same direction.
Transcriptional analysis (transcript mapping and promoter
localization) showed that the amt gene was transcribed in
a separate transcript (1.6 kb), and in a common transcript
with nha1 and nha2 (3 kb). In addition, a 1.5-kb transcript
covering the genes nha1 and nha2 was detected. Transcription
pattern seems therefore complex due to the presence
of promoters and terminators within the cluster. The regions
upstream of each gene were tested for their promoter activity
using transcriptional fusion with the gfpuv (green fluorescent
protein) reporter in R. erythropolis. Promoter of the amt gene
was localized by determination of the amt transcription start
point. Expression of both amidase and nitrile hydratase genes
was found to be constitutive according to RNA-hybridization,
transcriptional fusion measurements and enzyme activity
assays. The amt gene was cloned and expressed in
Escherichia coli and R. erythropolis using the pEXT20 and
pFX16 expression vectors, respectively.

PRODUCTION OF IMPROVED RHIZOBIAL STRAINS FOR
ENVIRONMENTAL STRESSES TOLERANCE AND NODULATING
BROAD -HOST RANGE PLANTS
Mohamed Mahmoud1, Mohamed Abd El-Halim1,
Samir Ibrahim2, Fatema Badawy2, Mohamed AboAbba1
1 Microbial Genetics Department, National Research Centre,
Cairo, Egypt.
2 Genetics Department, Faculty of Agriculture, Ain Shams
University, Cairo, Egypt.
Environmental stresses can dramatically affect the biological
niches of rhizobia-legumes symbioses. The present
investigation aimed to enhance and produce high efficient
rhizobial strains which could tolerate some heavy metals,
drought and salinity, and could nodulate broad host range
plants. Three indigenous Rhizobium leguminosarum biovar
 trifolii (Rt17, Rt17 and Rt11) and one Rhizobium meliloti (Rm2)
strains were subjected to protoplast fusion to gather certain
classified characters from each of them. Two attempts were
made where the first was between Rt7 and Rt1 H1 where no
fusants acquiring both parents tolerance were obtained which
may reflect the incompatibility between the parental
plasmids. In another attempt, Rt17 and Rm2 protoplasts were fused and
four fusants were isolated. All fusants had better performance
than the parental strains. They were resistant to each of the
antibiotics chloramphenical and ampicilcline, and the heavy
metals iron, cobalt, nickel and zinc. They have better salinity
tolerance (up to 7.5% NaCl), (up to 4.4 times the Rm2 and 1.4 times the Rt17),
drought tolerance (up to 6 times the Rt17 and two times the
Rm2). All fusants were able to nodulate both Egyptian
decor (as Rt17) and sweet decor (as Rm2). Their nodulation
mating efficiencies were improved 2 to 4.4 times more than the Rt17
and 1.3 to 2 times more than the Rm2 parental strains. All
fusants showed higher symbiotic efficiencies which resulted in